

The following Listing of the Claims will replace all prior versions and all prior listings of the claims in the present application:

Listing of The Claims:

1. (Currently Cancelled) An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.
2. (Currently Cancelled) The enzyme mixture of claim 1, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.
3. (Currently Cancelled) The enzyme mixture of claim 2, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, U1Tma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.
4. (Previously Cancelled) The enzyme mixture of claim 1, wherein said second enzyme is a mutant DNA polymerase.
5. (Previously Cancelled) The enzyme mixture of claim 4, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.
6. (Currently Cancelled) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.
7. (Previously Cancelled) An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

8. (Previously Cancelled) The enzyme mixture of claim 4, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

9. (Currently Cancelled) The enzyme mixture of claim 6, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

10. (Currently Cancelled) The enzyme mixture of claim 1 or 9, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

11. (Currently Cancelled) The enzyme mixture of claim 1, further comprising a PCR enhancing factor and/or an additive.

12. (Currently Cancelled) The enzyme mixture of claim 6, wherein said mutant Pfu DNA polymerase comprises a mutation in its partitioning domain or the polymerase domain.

13. (Currently Cancelled) A kit for DNA synthesis comprising a first enzyme, a second enzyme, and packaging material therefor, wherein said first enzyme comprises a DNA polymerization activity, said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

14. (Currently Cancelled) The kit of claim 13, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

15. (Currently Cancelled) The kit of claim 14, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

16. (Previously Cancelled) The kit of claim 15, wherein said second enzyme is a mutant DNA polymerase.

17. (Previously Cancelled) The kit of claim 16, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: Ultma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

18. (Previously Cancelled) The kit of claim 17, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

19. (Currently Cancelled) A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

20. (Previously Cancelled) A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a Taq DNA polymerase, and packaging material therefore, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

21. (Currently Cancelled) The kit of claim 13 or 19, further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or an additive, a control DNA template and a control primer.

22. (Currently Cancelled) The kit of claim 19, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

23. (Currently Cancelled) The kit of claim 13 or 22, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E,

Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

24. (Previously Withdrawn from Consideration) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a DNA polymerization activity, and a second enzyme which is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

25. (Previously Withdrawn from Consideration) The method of claim 24, wherein said nucleic acid template is a DNA molecule.

26. (Previously Withdrawn from Consideration) The method of claim 25, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

27. (Previously Withdrawn from Consideration) The method of claim 26, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, Ultma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

28. (Previously Cancelled) The method of claim 25, wherein said second enzyme is a mutant DNA polymerase.

29. (Previously Cancelled) The method of claim 28, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: Ultma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

30. (Previously Cancelled) The method of claim 28, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

31. (Previously Withdrawn from Consideration) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

32. (Previously Withdrawn from Consideration) A method for TA cloning of DNA synthesis product comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and

(c) inserting said synthesized DNA product into a TA cloning vector.

33. (Previously Withdrawn from Consideration) The method of claim [29, ]31, or 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

34. (Previously Withdrawn from Consideration) The method of claim 24 [33], wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

35. (Previously Withdrawn from Consideration) The method of claim 24, 31 or 32, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

36. (Previously Withdrawn from Consideration) An isolated mutant DNA polymerase comprising a reduced DNA polymerization activity.
37. (Previously Withdrawn from Consideration) An isolated mutant DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.
38. (Previously Withdrawn from Consideration) The mutant DNA polymerase of claim 36 or 37, wherein said mutant DNA polymerase comprises a mutation in the partitioning domain or in the polymerase domain.
39. (Previously Withdrawn from Consideration) The mutant DNA polymerase of claim 37, wherein said mutant DNA polymerase is a mutant Pfu DNA polymerase.
40. (Previously Withdrawn from Consideration) A mutant Pfu DNA polymerase with reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.
41. (Previously Withdrawn from Consideration) The mutant DNA polymerase of claim 40, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
42. (Previously Withdrawn from Consideration) The mutant DNA polymerase of claim 37, wherein said mutant DNA polymerase is derived from the group consisting of: U1Tma DNA polymerase, Tli DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.
43. (Previously Withdrawn from Consideration) A composition for DNA synthesis comprising an isolated mutant DNA polymerase which comprises a reduced DNA polymerization activity.
44. (Previously Withdrawn from Consideration) A composition for DNA synthesis comprising an isolated mutant DNA polymerase which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

45. (Previously Withdrawn from Consideration) The composition of claim 43 or 44, wherein said mutant DNA polymerase comprises a mutation in the partitioning domain or the polymerase domain.

46. (Previously Withdrawn from Consideration) The composition of claim 44, wherein said mutant DNA polymerase is a mutant Pfu DNA polymerase.

47. (Previously Withdrawn from Consideration) A composition comprising a mutant Pfu DNA polymerase, wherein said mutant DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

48. (Previously Withdrawn from Consideration) The composition of claim 47, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

49. (Previously Withdrawn from Consideration) The composition of claim 44, wherein said mutant DNA polymerase is derived from the group consisting of: Ultma DNA polymerase, Tli DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

50. (Previously Withdrawn from Consideration) A kit for DNA synthesis comprising a mutant DNA polymerase which comprises a reduced DNA polymerization activity and packaging material therefore.

51. (Previously Withdrawn from Consideration) A kit for DNA synthesis comprising a mutant DNA polymerase which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity and packaging material therefore.

52. (Previously Withdrawn from Consideration) The kit of claim 50 or 51, wherein said mutant DNA polymerase comprises a mutation in the partitioning domain or the polymerase domain.

53. (Previously Withdrawn from Consideration) The kit of claim 51, wherein said mutant DNA polymerase is a mutant Pfu DNA polymerase.

54. (Previously Withdrawn from Consideration) A kit comprising a mutant DNA polymerase which comprises a reduced DNA polymerization activity and packaging material therefor, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

55. (Previously Withdrawn from Consideration) The kit of claim 54, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

56. (Previously Withdrawn from Consideration) The kit of claim 51, wherein said mutant DNA polymerase is derived from the group consisting of: Ultma DNA polymerase, Tli DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

57. (Previously Withdrawn from Consideration) A mutant Pfu DNA polymerase produced by introducing a mutation into a polynucleotide encoding a wild type Pfu DNA polymerase to produce a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

58. (Previously Withdrawn from Consideration) A mutant Pfu DNA polymerase comprising a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase is produced by the steps:

(a) providing a polynucleotide encoding a wild-type Pfu DNA polymerase;

(b) introducing one or more nucleotide mutations into said polynucleotide to produce a mutant polynucleotide encoding said mutant Pfu DNA polymerase; and

(c) expressing said mutant polynucleotide to produce said mutant Pfu DNA polymerase, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

59. (Previously Withdrawn from Consideration) The mutant DNA polymerase of claim 58, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

60. (Previously Withdrawn from Consideration) A composition comprising a mutant Pfu DNA polymerase produced by expressing a polynucleotide encoding a Pfu DNA polymerase with a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

61. (Previously Withdrawn from Consideration) A composition comprising a mutant Pfu DNA polymerase comprising a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase is produced by the steps:

(a) introducing a mutation into a polynucleotide encoding a wild-type Pfu DNA polymerase to produce said mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388;

(c) expressing said mutant polynucleotide to produce said composition comprising said mutant Pfu DNA polymerase.

62. (Previously Withdrawn from Consideration) The composition of claim 60 or 61, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

63. (Previously Withdrawn from Consideration) The method of claim 33, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

64. (Currently Added) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is an Archaeal DNA polymerase, said second enzyme is a mutant

Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein the mutant Archaeal DNA polymerase comprises a mutation selected from the group consisting of amino acid positions corresponding to D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388 of Pfu DNA polymerase.

65. (Currently Added) The enzyme mixture of claim 64, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: Tli DNA polymerase (Vent DNA polymerase), Deep Vent DNA polymerase, Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, and JDF-3 DNA polymerase.

66. (Currently Added) The enzyme mixture of claim 65, wherein said mutant DNA polymerase comprises a mutation in its partitioning domain or polymerase domain.

67. (Currently Added) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein said mutant DNA polymerase comprises a mutation at a position as indicated in Tables 2A and 2B.

68. (Currently Added) The enzyme mixture of claim 67, wherein said mutant DNA polymerase comprising a mutation in its partitioning domain or polymerase domain is a mutant Pfu DNA polymerase, KOD DNA polymerase, or JDF-3 DNA polymerase.

69. (Currently Added) The enzyme mixture of claim 68, wherein said mutant Pfu DNA polymerase contains a mutation at an amino acid position selected from the group consisting of D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

70. (Currently Added) The enzyme mixture of claim 69, wherein said mutant Pfu DNA polymerase contains a mutation of G387P.

71. (Currently Added) The enzyme mixture of claim 68, wherein said mutant KOD DNA polymerase contains a mutation at an amino acid position selected from the group consisting of Y384, G386, G387, D404, T541, D542, and K592.

72. (Currently Added) The enzyme mixture of claim 71, wherein said mutant KDO DNA polymerase contains a mutation of G387P.

73. (Currently Added) The enzyme mixture of claim 68, wherein said mutant JDF-3 DNA polymerase contains a mutation at amino acid position G387.

74. (Currently Added) The enzyme mixture of claim 73, wherein said mutant JDF-3 DNA polymerase contains a mutation of G387P.

75. (Currently Added) The enzyme mixture of claim 64, wherein said first enzyme and said second enzyme are derived from two different Archaeal DNA polymerases.

76. (Currently Added) The enzyme mixture of claim 75, wherein said first enzyme is wild type KOD or wild type JDF-3 DNA polymerase, and said second enzyme is a mutant Pfu DNA polymerase.

77. (Currently Added) The enzyme mixture of claim 76, wherein said mutant Pfu DNA polymerase contains a mutation at amino acid G387.

78. (Currently Added) The enzyme mixture of claim 77, wherein said mutant Pfu DNA polymerase contains a mutation of G387P.

79. (Currently Added) The enzyme mixture of claim 75, wherein said first enzyme is wild type Pfu DNA polymerase, and said second enzyme is a mutant KOD or mutant JDF-3 DNA polymerase.

80. (Currently Added) The enzyme mixture of claim 79, wherein said mutant KOD or mutant JDF-3 DNA polymerase contains a mutation of G387.

81. (Currently Added) The enzyme of claim 80, wherein said mutant KOD or mutant JDF-3 DNA polymerase contains a mutation of G387P.

82. (Currently Added) The enzyme mixture of claim 67, wherein said first enzyme is Taq DNA polymerase.

83. (Currently Added) The enzyme mixture of claim 82, wherein said second DNA polymerase is a mutant Pfu, a mutant KOD or a mutant JDF-3 DNA polymerase.

84. (Currently Added) The enzyme of claim 83, wherein said mutant Pfu, mutant KOD, or mutant JDF-3 DNA polymerase contains a mutation of G387P.

85. (Currently Added) A kit comprising an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is an Archaeal DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein the mutant Archaeal DNA polymerase comprises a mutation selected from the group consisting of amino acid positions corresponding to D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388 of Pfu DNA polymerase, and packaging material therefor.

86. (Currently Added) A kit comprising an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein said mutant DNA polymerase comprises a mutation at a position as indicated in Tables 2A and 2B, and packaging material therefor.

87. (Currently added) The kit of claim 85 or 86, further comprising a reagent selected from the group consisting of: dNTPs, reaction buffer, primer, and DNA enhancing factor.